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Malva sylvestris extract alleviates the astrogliosis and inflammatory stress in LPS-induced depression mice

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ABSTRACT

Neuro-inflammation is widely regarded as the inflammation occurred in the central nervous system (CNS) tissue, which authentically involved in the pathogenesis such as depression although the underlying mechanism remains to be elucidated. *Malva sylvestris* (MS), a plant widely used in traditional medicine to mitigate urological, respiratory and oral diseases, exhibits excellent anti-oxidative and anti-inflammatory properties. In the present study, we first used LPS-induced depression-like mice to evaluate the neuro-protective effect of MS extract. We found that, after 7 days' administration of MS extract, the cognitive impairment of LPS-induced depression-like mice was efficiently alleviated, evaluated by behavioral test including the Open field, Morris water maze (MWM), Elevated plus-maze (EPM) and Rota-rod test. Furthermore, we found that MS extract also inhibited the LPS-induced neuron apoptosis and astrogliosis both in the cortex and the CA1 region of hippocampus. Finally, our findings showed that the extract of MS relieved inflammatory stress induced by LPS injury, indicated by the down-regulation of IL-1β/6 and TNF-α, and up-regulation of IL-4 level both *in vitro* and *in vivo*. Collectively, MS extract exhibits neuro-protective activity *in vivo*, and therefore, it may be widely used for food to relieve the symptoms of neuro-inflammation associated disorders such as depression.

1. Introduction

Neuro-inflammation is widely regarded as the inflammation occurred in the central nervous system (CNS) tissue, manifesting the sustained activation of glial cells such as microglia and astrocytes in the brain tissue (Gendelman, 2002; Kyritsis et al., 2014; Sofroniew, 2015). The extensive activation of glial cells may directly damage the surrounding neurons by creating inflammatory stress (TNF-α, IL-1β and IL-6) and oxidative stress (nitric oxide and reactive oxygen species), and ultimately lead to the neuron apoptosis and the dysfunction of CNS (Belarbi et al., 2012; Tai et al., 2013). Although the underlying mechanisms remain to be elucidated, neuro-inflammation is authentically involved in the pathogenesis of both Alzheimer's disease (AD), depression, multiple sclerosis and ischemic stroke (Block and Calderon-Garciduenas, 2009; Colombo and Farina, 2016; Felts et al., 2005; Pekny and Pekna, 2014; Walker et al., 2014). In 2013, Tai and colleagues observed that FLZ, a natural squamosamide derivative from a Chinese herb, exerts neuro-protection in lipopolysaccharide (LPS)-induced depression-like animals through its anti-inflammatory effect. Therefore, the medicines exhibiting anti-inflammatory activity may be potential candidates for the treatment of neuro-inflammation associated disorders in human.

Malva sylvestris (MS), also known as common mallow or “Jinkui” in Chinese, is widespread across Asia, North Africa and Europe (Gasparetto et al., 2012). It is outstanding among the numerous plants used in traditional medicine due to its various applications including gastrointestinal disturbance, dermatological ailments, menstrual pains, urological disorders, respiratory diseases and oral diseases (Gasparetto et al., 2012). In chemical composition, MS plants mainly contain abundant flavonoids which exhibit excellent anti-oxidative and anti-inflammatory activity (Barros et al., 2010). Indeed, numerous recent studies have demonstrated that, by releasing oxidative and inflammatory stress, the extracts of MS protect liver, heart and kidneys from ischemia/reperfusion (I/R)-induced injury in animals (Martins et al., 2017; Prudente et al., 2013; Saad et al., 2017; Zuo et al., 2017). Recently, Mohamadi and colleagues unveiled that MS extract also reduces cisplatin-induced renal and hepatic side effects depending on its anti-inflammatory activity (Mohamadi Yarijani et al., 2018). However, whether MS is effective in neuro-inflammation associated disorders such as depression is not fully investigated.

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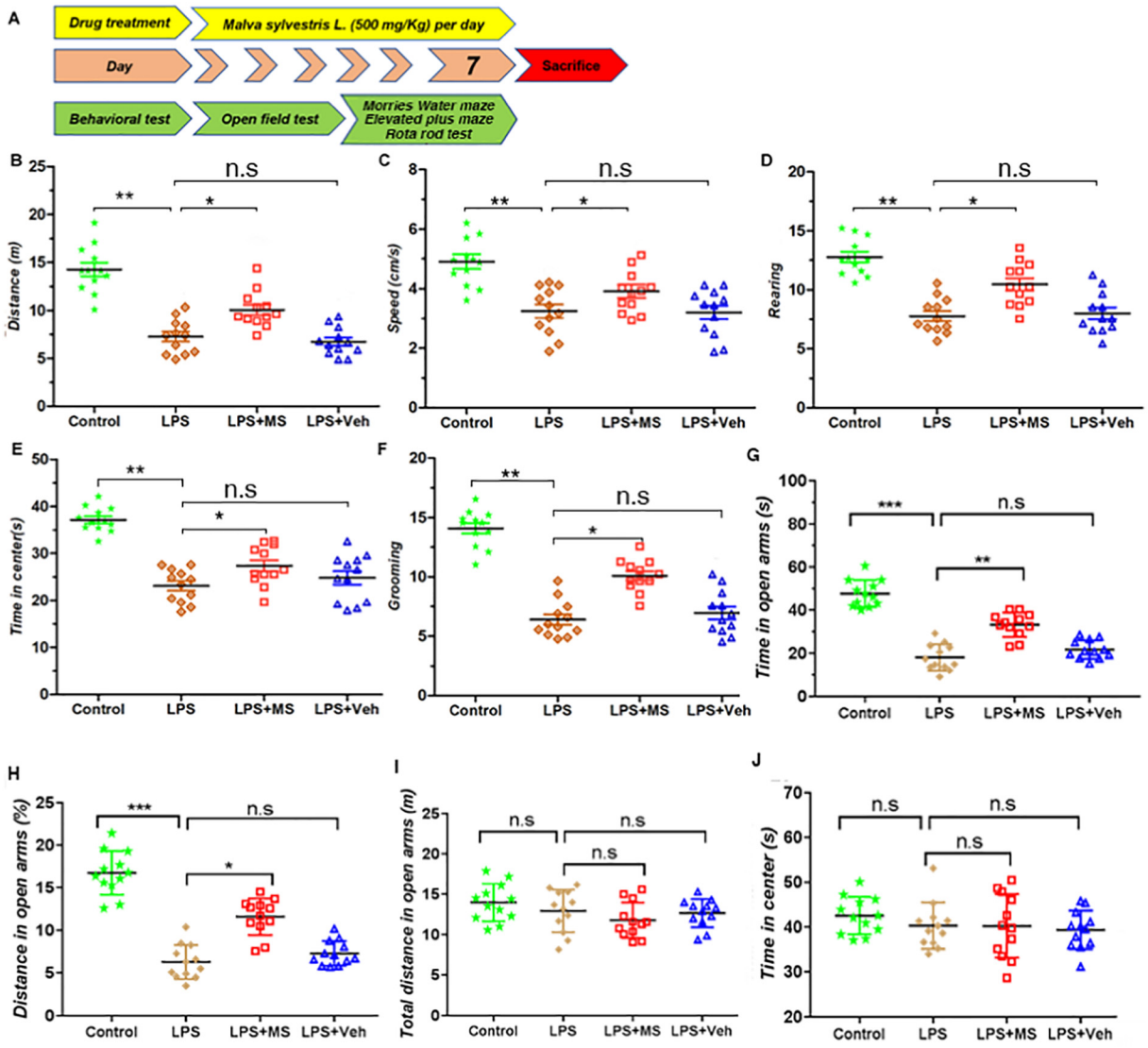

Fig. 1. MS extract improves LPS-induced anxiety in mice.
(A) The schedule of drug treatments and behavioral test.
(B—F) The open field test evaluates the locomotor activity, anxiety, and willingness to explore of animals in each group. The moving distance (B) and time (E) in the center of the open field, moving speed (C), and rearing (D)/grooming (F) frequency were monitored by the camera. MS, *Malva sylvestris* extract; Veh, vehicle. $n = 12$; $^{*}p < .05$; $^{**}p < .01$; n.s, no significance in statistic.
(G-J) The Elevated plus maze test estimates the anxiety of animals in each group. The time (G), moving distance (H) and the total time (I) in open arms, and time in center (J) of experimental equipment were recorded. n = 12; *p < .05; **p < .01; ***p < .01; n.s, no significance in statistic.

In the present study, we first evaluated the neuro-protective activity of MS extract in LPS-induced depression-like mice. By behavioral test including the Open field, MWM, EPM and Rota-rod test, we found that MS markedly alleviated the cognitive impairment induced by LPS treatment. Subsequently, we observed that MS extract inhibited the LPS-induced neuron apoptosis and astrogliosis both in the cortex and the CA1 region of hippocampus. Finally, our results showed that the extract down-regulated the elevated IL-1β/6 and TNF-α concentration induced by LPS injury both *in vitro* and *in vivo*.

2. Materials and methods

2.1. Animals and treatment protocol

In the present study, healthy adult SPF-grade mice were used for animal experiments. All animals were purchased from the animal center of the Xuzhou Medical University, and were kept in a pathogen-free animal house (with a 12 h dark/12 h light cycle) at room temperature (about 25 ± 1 °C), supplying adequate food and water. All experimental animals were maintained in accordance with the Guide for the Care and Use of Laboratory Animals, and the protocol was approved by the Ethics Committee of the Xuzhou Medical University. The operations were performed under general anesthesia to minimize the suffering of animals.

Prior to the injection of LPS, the anti-inflammatory sulindac sulfide (Sigma-Aldrich, USA) was given orally at a concentration of 3.75 or 7.5 mg/kg for > 3 weeks. The animals were randomly divided into four groups ($n = 12$ per group). 1) Normal control group received 0.9% saline *via* the intraperitoneal (i.p.) route; 2) LPS group were injected with 250 μg/kg of lipopolysaccharide (LPS, Sigma-Aldrich, USA) *via* i.p. one day before treatment; 3) MS treated group received MS extract (250 mg/kg) intragastrically per day for the duration of seven consecutive days; 4) Vehicle control group received an equal volume of vehicle.

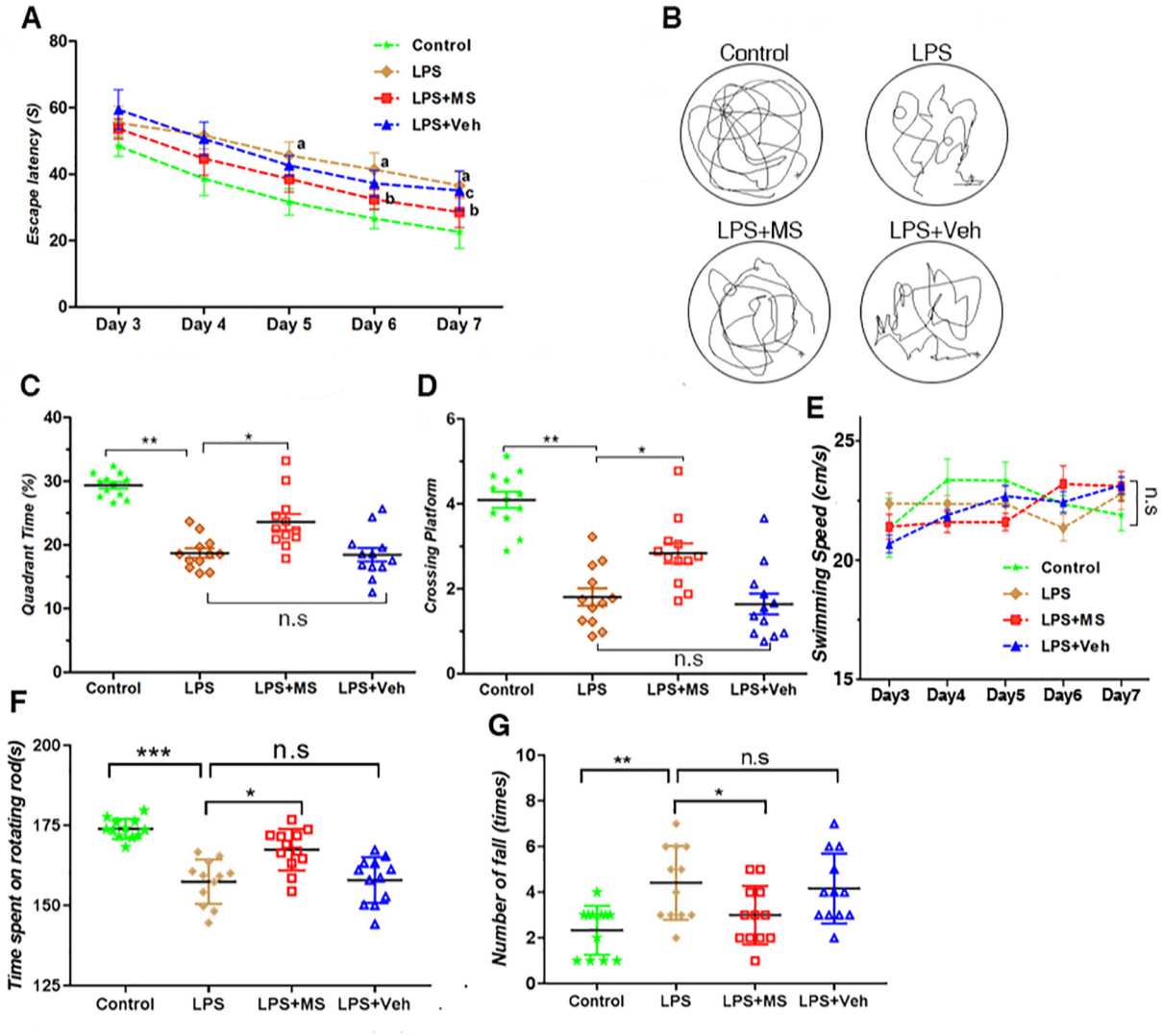

Fig. 2. MS extract mitigates LPS-induced impairment of spatial memory ability in mice.
(A-E) The MWM test evaluates the learning and spatial memory ability of animals in each group. The escape latency (A), moving track (B), the time in target quadrant (C), the times of crossing with platform place (D), and the swimming speed of animals (E) were analyzed. In A, $p^a < 0.05$, LPS group *vs.* control group; $p^b < 0.05$, LPS + MS group *vs.* LPS group; $p^c < 0.05$, LPS + Veh group *vs.* LPS group. n = 12; *p < .05; **p < .01; n.s, no significance in statistic.
(F-G) The Rota-rod test evaluates the motor coordination ability of animals in each group. The time spent on the rotating rod (F) and the total number of fall (G) of each animal were recorded. n = 12; *p < .05; **p < .01; ***p < .001; n.s, no significance in statistic.

2.2. Preparation of Malva sylvestris extract

The MS extract was prepared following the previous report (Qin et al., 2017). Whole plants of MS were collected and authenticated by three independent professors from the Botanical Department of the Southern Medical University, China. The leaves and flowers of MS plant were washed, air dried, cut into small pieces, and finally ground into powder using a grinder. The powder was macerated in 95% methanol for a week. The extract was filtered out using 22-μm filter, and the filtrate was dried using a rotary evaporator. The final extract was directly used for animal experiment at the dose of 250 mg/kg.

2.3. Behavioral test

2.3.1. Open field test

To evaluate the locomotor activity of animals, the open field test was performed following the method previously described by Cunha and Masur (1978). The animals were placed in an open wooden box (80 × 80 × 40 cm) for 5 min, of which the walls were red and the white floor were divided into16 equal squares (4 × 4) by black lines. The behavior of animals was monitored by camera.

2.3.2. Morris water maze (MWM)

Morris water maze (MWM) test was conducted following the previously reported protocol with subtle modification (Khallaf et al., 2017; Qin et al., 2017), using the SMART-CS (Panlab, Barcelona, Spain) program and equipment. A circular pool (diameter: 1.2–2 m) containing water (25 ± 1 °C) to a depth of 35 cm and a hidden platform (~1.0 cm below the water) was used in the present study. During the training, each animal was placed into the water facing the pool wall and was allowed to find the platform within 1 min. The mouse who successfully reached the platform in due time was allowed to rest for 15 s on the platform, and the one who cannot reach the platform in due time was moved manually to the platform to rest for 15 s. Subsequently, the hidden platform was removed for the probe test. Each animal was placed at the opposite location of the previous one in the same pool. Escape latency, swimming speed and track of each animal were monitored using a video camera for 3 days (1 time/day).

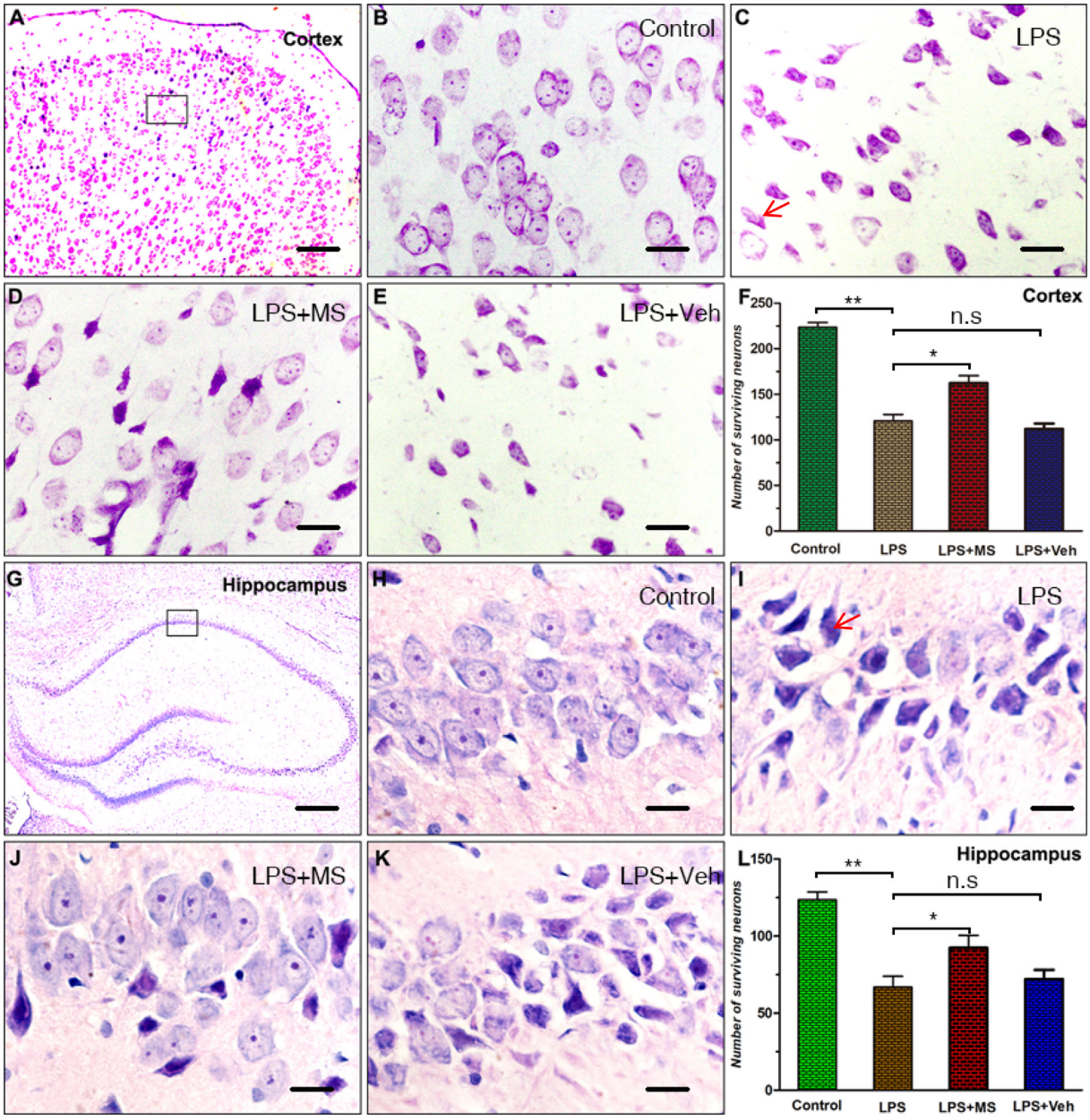

Fig. 3. MS extract inhibits LPS-induced neuron apoptosis in CA1 region of hippocampus.
(A-L) TUNEL shows the apoptotic neurons in cortex and CA1 region of hippocampus of each group. (A-E) the cortex region of mouse; scale bar in A, 200 μm and in B-E, 20 μm. (F) the quantification of surviving neurons in B-E. (G-K) the hippocampus region of mouse; scale bar in A, 200 μm and in B-E, 20 μm (L) the quantification of surviving neurons in H–K. The red arrows point the apoptotic neurons. $n = 6$; *p < .05; **p < .01; n.s, no significance in statistic. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

2.3.3. Elevated plus-maze (EPM)

Elevated plus-maze (EPM) test was conducted in accordance with the previous reports (Andrade et al., 2003), using an open wooden box with two open arms (50 × 10 cm, 3 mm wood rim) perpendicular to two closed arms (50 × 10 × 40 cm) both elevated 50 cm from the floor. The animals were placed at the center of the plus-maze facing the closed arms to adapt for 5 min, and then the test was performed with duration of 5 min. The illumination intensity of the experimental room was at 25 ± 1 °C, and the animals' behavior was monitored using a video camera without experimenters staying aside. After each test, the arms were thoroughly cleaned with ethanol solution.

2.3.4. Rota-rod test

Rota-rod test was performed following the previous report with subtle modification (Chen et al., 2018). Briefly, the animals were trained to remain for 180 s on a rotating rod with an angular speed of 17 rpm before testing. Subsequently, each animal received a testing of 180 s. During the testing, the animals were placed on the rotating bar with a diameter of 2.5 cm, which was elevated 25 cm above the floor. The number of falls and the time of spent on the bar of each animal were recorded. After each trial, the apparatus was thoroughly cleaned with ethanol solution.

2.4. Tissue preparation

After 24 h of the behavioral tests, animals were euthanized by diethyl ether, and then the brain tissues were immediately removed in ice-cold saline and fixed using 10% formalin solution for the further

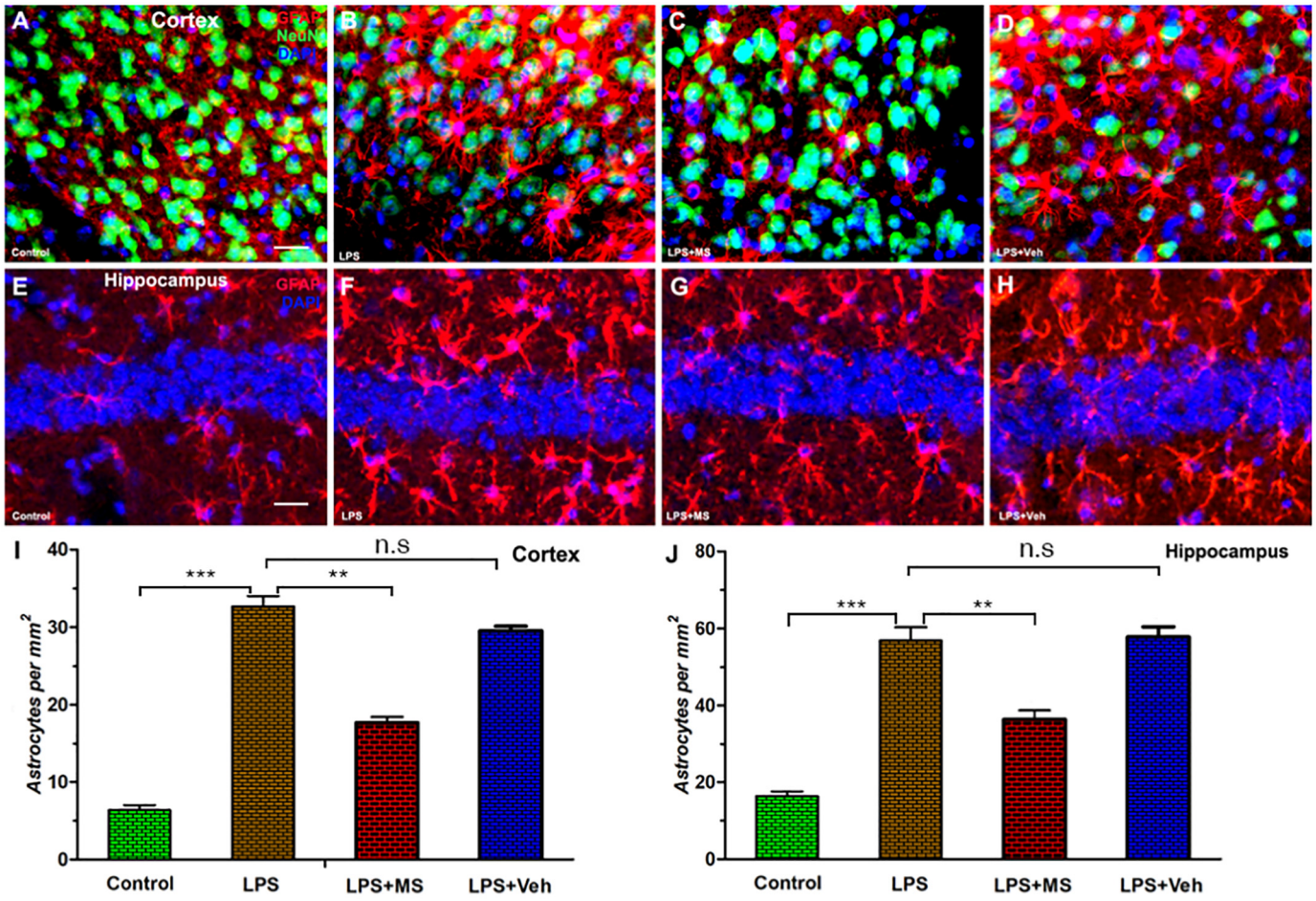

Fig. 4. MS extract reactivates astrocytes in LPS-injured brain tissue.
(A-H) GFAP staining shows the astrogliosis. The neurons were labeled by anti-NeuN. (A-D) the cortex region of mouse, (*E*-H) the hippocampus region of mouse. (I-J) The quantification of astrocytes in A-D (I) and E-H (J). Scale bar, 50 μm; n = 6; **p < .01; ***p < .001; n.s, no significance in statistic.

histopathological examination. As for the determination of cytokines content, the brain tissues were homogenized in cold lysis buffer. After centrifuged, the supernatant was withdrawn carefully for further examination or keeping in −80 °C for a long time.

2.5. Terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL)

To determine the neurons apoptosis, the commercial TUNEL reaction kit (Roche Diagnostics GmbH, Mannheim, Germany) was used for tissue sections staining. The experiment was performed following the manufacturer's instruction. Briefly, the sections were incubated using the TUNEL reaction buffers in a dark humidified chamber for 1 h at 37 °C, followed by a final wash using PBS for 3 × 10 min and then covered using water-based mounting medium (National Diagnotics, Atlanta, USA).

2.6. Immunofluorescence staining

Before stained, the slides were gently washed 3 × 5 min using PBS, followed by punching the cell membrane using 0.2% Trion X-100/PBS at room temperature for 15–20 min. After brief washing using PBS, the slides were incubated in blocking solution (containing 3–5% BSA/PBS) for 1 h. After blocking, the slides were incubated using the primary antibodies including anti-GFAP (MAB360, Sigma-Aldrich, USA) and anti-NeuN (A60, Millipore, Germany) overnight at 4 °C. The next day, the slides were washed using PBS for 3 × 5 min, and then incubated with Rhodamine/FITC-labeled primary antibodies (Santa Cruz Biotechnology, USA) and Hoechst (Sangon Biotech, China) for 1 h at room temperature. The slides were mounted using Prolong Anti-fade Reagent (Thermo Fisher Scientific, USA). Slides were examined using a confocal laser-scanning microscope (Flouview FV 1000, Olympus, Japan), and the photos were analyzed using ImageJ software.

2.7. Cell culture

BV-2 cells were purchased from Cell Bank of the Chinese Academy of Sciences (Shanghai, China). BV-2 cells were cultured in F12 DMEM (Gibco, Grand Island, NY, USA) with 10% fetal bovine serum (FBS, Gibco, USA) and 1% penicillin/streptomycin (Gibco, Grand Island, USA), and maintained in a 5% CO_2 humidified incubator (at 37 °C). To evaluate the anti-inflammatory functions of MS extract *in vitro*, BV-2 cells were seeded in 12-well plate (3×10^5 per well) and treated using MS extract at the indicated concentration with/without LPS (100 ng/ml) for 12 h. Then, the supernatant was collected for determining inflammatory cytokine concentration.

2.8. ELISA assay to determine inflammatory cytokines

To determine the content of cytokines in the brain tissue homogenate, including IL-1β, IL-4/6 and TNF-α, the commercial ELISA kits (Thermo Scientific™, USA) were used. The experiments were conducted following the manufacturer's instruction.

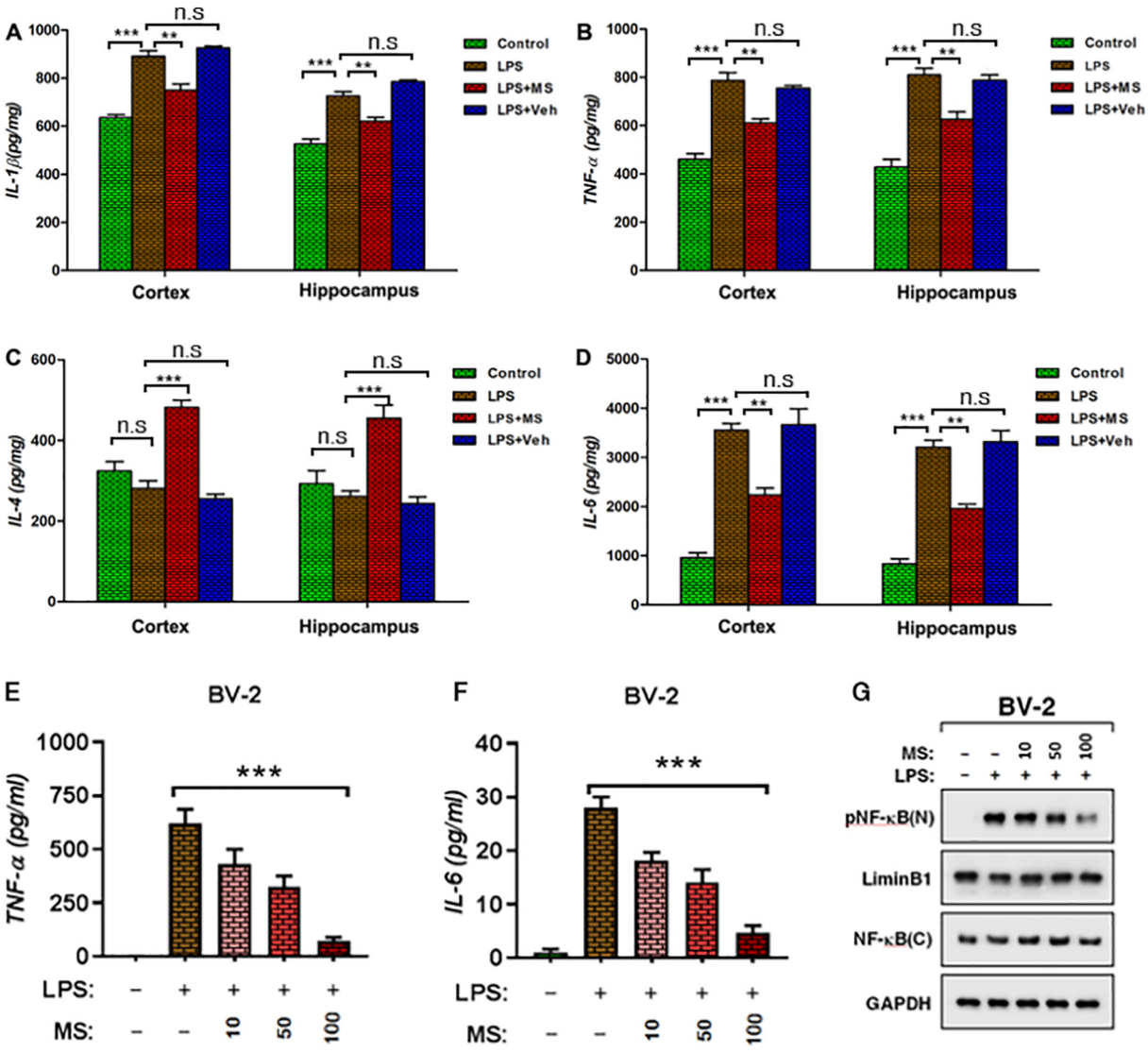

Fig. 5. MS extract releases the inflammatory stress in LPS-injured brain tissue.
(A-D) ELISA assay determines the concentration of cytokines in brain tissue of each group. (A) IL-1β, (B) TNF-α, (C) IL-4 and (D) IL-6. n = 6; **p < .01; ***p < .001; n.s, no significance in statistic.
(E-F) ELISA assay determines the concentration of cytokines the supernatant of each BV-2 cell. The cells were seeded in 12-well plate (3×10^5 per well) and treated using MS extract at the indicated concentration with/without LPS (100 ng/ml) for 12 h. (E) TNF-α and (F) IL-6 in supernatant of BV-2 cells were determined. $n = 9$; ***$p < .001$.
(G) Western blotting determines the indicated protein level in BV-2 cells. The cell lysates in E were used. N, nucleus; C, cytoplasm. LiminB1 and GAPDH serve as nuclear and cytoplasmic loading control, respectively.

2.9. Western blotting

After removed the supernatant, BV-2 cells were lysed using 1 × sample buffer containing 50 mM Tris pH 6.8, 2% SDS, 0.025% Bromophenol blue, 10% glycerol and 5% BME. Protein samples were separated using 7.5–10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), and transferred to nitrocellulose (NC) membrane. After blocked using 5% skim milk/TBST, the membranes were incubated with different primary antibodies at 4 °C overnight, including anti-NF-κB, anti-pNF-κB, anti-LiminB1 and anti-GAPDH purchased from Cell Signaling Technology (Beverly, USA). The next day, the membranes were incubated using the HRP-conjugated secondary antibodies for 1 h at room temperature. After washing using 1 × TBST for 3 × 5 min, the proteins were detected using Tanon™ High-sig ECL Western Blotting Substrate Kit (Tanon, China) in accordance to manufacturer's instruction, and finally scanned and analyzed using a Bio-Rad ChemiDoc MP (Bio-Rad, USA).

2.10. Statistical analysis

In the present study, the results were expressed as mean ± SD, and duplications of each experiment was specified in the corresponding legend. The Graphpad prism7 software was used for statistical analysis. Student's *t*-test was used for the mean comparison between two groups. Two-way ANOVA was used for multiple group comparison followed by Bonferron correction *post hoc* test. $P < .05$ was considered as statistical significance.

3. Results

3.1. MS extract alleviates LPS-induced anxiety in mice

In this study, to evaluate the neuro-protective effect of MS extract, we took full advantage of the LPS-induced depression-like mice, a widely used neuro-inflammation animals (Lee et al., 2008; Zhao et al.,

2019a). We strictly followed the schedule of drug treatments and behavioral test shown in Fig. 1A. Firstly, to estimate the general locomotor activity, anxiety, and willingness to explore of animals, we performed the open field test, a common animal behavioral experiment (Khallaf et al., 2017). Indeed, we found that, in LPS group, the distance/time in center of the open field, moving speed and rearing/grooming frequency were significantly decreased compared with those of control group (Fig. 1 B—F), suggesting LPS treatment injures animals' locomotor activity and willingness to explore, and enhances their anxiety. Intriguingly, these detrimental effects were markedly ameliorated after MS extract administration but not vehicle (Fig. 1B-F). Furthermore, we evaluated the anxiety of mice after MS administration using Elevated plus-maze (EPM) test. Consistently, we found that the animals of LPS + MS group spent more time and moved longer distance in the open arms compared with LPS group without distinguishable difference in total moving distance and time in center of EPM equipment (Fig. 1G-J), suggesting MS extract ameliorates the anxiety of mice induced by LPS treatment. These observations indicate that MS extract alleviates LPS-induced anxiety in mice.

3.2. MS extract mitigates LPS-induced impairment of spatial memory ability in mice

Subsequently, we performed the Morris water maze (MWM) test to evaluate the learning and spatial memory ability of animals. As indicated in Fig. 2A, in the acquisition trial, the escape latency of animals in LPS group increased significantly since the 5th day of the test, indicating the learning ability of the brain of LPS group is abated. Interestingly, MS extract administration markedly reversed the functional lesion of the brain since the 6th day of the test, which was obviously more efficient than vehicle treatment (Fig. 2A). In the spatial probe test, the animals of LPS group spent shorter time in the target quadrant and crossed less times with the target place compared with that of control group (Fig. 2B-D). Intriguingly, the performances of MS extract treated group were remarkably better than those of LPS group, being very close to those of control group (Fig. 2B-D). Besides, the LPS/MS treatment did not affect the moving ability of animals, indicated by the undistinguishable swimming speed (Fig. 2E). Finally, we observed that MS extract dramatically improved the motor coordination ability of LPS-injured animals in a Rota-rod test (Fig. 2F and G). Collectively, our findings show MS extract administration improves the brain functions of LPS-injured mice.

3.3. MS extract inhibits LPS-induced neuron apoptosis

The learning and spatial memory ability of the brain is mainly responsible by the cortex and hippocampus of brain respectively (Lykhmus et al., 2016). Extensive previous studies indicated that the loss of neurons in the cortex and CA1 region of hippocampus usually causes the impairment of brain function (Bird, 2017; Norman, 2010). Therefore, we histochemically examined the brain tissues using Terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL), an extensively used method to label apoptotic cells *in situ* (Gavrieli et al., 1992). Indeed, we found that the neurons in cortex lost noticeably after LPS injury, and however, this toxicity of LPS on neurons was substantially alleviated by MS extract administration, but not vehicle (Fig. 3A-F). Consistently, the similar results were observed in the CA1 region of hippocampus (Fig. 3G-L). Altogether, our data suggest that MS extract exhibits neuro-protective effects on LPS-injured brain.

3.4. MS extract inhibits in LPS-induced astrogliosis

Astrogliosis, also named as astrocytosis, is an abnormal increase in the number of astrocytes due to the destruction of nearby neurons from CNS (Sofroniew, 2014). Previously, LPS-induced astrogliosis has been extensively observed by many dependent groups both *in vitro* and *in vivo* (Lee et al., 2008; Pekny and Pekna, 2014; Zhao et al., 2019b). In the present study, we also observed that, both in the cortex and hippocampus of the LPS-injured mice, the number of astrocytes increased by several folds compared with that of control group (Fig. 4A, B, E, F, I and J), manifesting the phenotype of astrogliosis. After 7 days' administration of MS extract, but not the vehicle, the elevated astrocyte number was significantly down-regulated both in cortex and hippocampus tissue although they were still more than that of control group (Fig. 4A, C, D, E, G, H, I and J). These observations suggest that MS extract alleviates the LPS-induced astrogliosis.

3.5. MS extract releases the inflammatory stress in LPS-injured brain tissue

Accumulating evidences demonstrate that MS extract exhibits anti-inflammatory bioactivity both in cell and animal model (Benso et al., 2016; Martins et al., 2017; Mohamadi Yarijani et al., 2018; Prudente et al., 2013; Qin et al., 2017). Therefore, we also evaluated the anti-inflammatory effect of MS extract on LPS-injured mice by detecting the concentration of inflammatory (IL-1β/6 and TNF-α) and anti-inflammatory (IL-4) factors in brain tissues. After LPS injury, the concentration of IL-1β/6 and TNF-α both in cortex and hippocampus were obviously up-regulated (Fig. 5A, B, and D), suggesting the inflammatory stress is established. The MS extract administration, but not vehicle, reduced the elevated IL-1β/6 and TNF-α concentration, and meanwhile increased IL-4 concentration both in cortex and hippocampus (Fig. 5A-D). These observations were further validated by our *in vitro* results in BV-2 cells, a widely used immortalized mouse microglial cell line. As shown in Fig. 5E-F, MS extract obviously inhibited the elevated IL-6 and TNF-α content induced by LPS treatment in BV-2 cells in a dose dependent manner. Furthermore, we found that MS extract down-regulated the phosphorylation level of NF-κB (in nucleus) induced by LPS treatment in BV-2 cells (Fig. 5G), suggesting the extract inactivates NF-κB signaling *in vitro*. Collectively, our findings indicate that MS exhibits considerable anti-inflammatory activity both *in vitro* and *in vivo*.

4. Discussion & conclusion

Malva sylvestris, as an edible and medicinal plant, has been consumed for thousands of years to treat, alleviate or prevent many human diseases in worldwide (Prudente et al., 2013). Nowadays, extensive studies involved in the chemical composition of MS have revealed that the flavonoids in its leaves and flowers are the main bioactive ingredients, which exhibit good anti-oxidative and anti-inflammatory properties (Benso et al., 2015; Gasparetto et al., 2012). Therefore, it is crucial to investigate the medicinal value of MS and the underlying molecular mechanism on an animal model for human diseases. In the present study, we evaluated the therapeutic properties of MS extract on LPS-induce depression-like mice for the first time, and found it noticeably alleviated the cognitive impairment induced by LPS administration using open field and MWM test. Our findings imply that using MS for food may mitigate the symptoms of depression patients.

The neuron apoptosis is a typical hallmark of neuro-inflammation associated disorders and neurodegenerative diseases (Villa et al., 2016; Walker et al., 2014). Indeed, in the present study, we also observed that the neuron apoptosis both in cortex and hippocampus were induced in LPS injured mice. After 7 days' administration of MS extract, the neuron apoptosis was obviously abolished, suggesting the extract shows excellent anti-apoptotic activity *in vivo*. In fact, Qin and colleagues recently reported that MS extract significantly decreased the count of neuro-degeneration in the CA1 and CA3 region of hippocampus of the mild traumatic brain injury (MTBI) rats model (Qin, Qin et al., 2017). These evidences collectively indicate that MS extract can exert its anti-apoptotic function in a context independent manner, suggesting it may have a wide application in the therapeutics of neuro-inflammation associated disorders.

Astrogliosis always occurs when astrocytes are in response to all

forms and severities of CNS injury and disease, which has been identified as a contributor to, or the primary causes of, inflammatory and oxidative stress (Lykhmus et al., 2016; Sofroniew, 2014). In our LPS-induced depression-like mice, we also observed that the astrogliosis was occurred in the cortex and CA1 region of hippocampus using GFAP staining, and intriguingly, astrogliosis was markedly ameliorated after MS extract administration. To further confirm these observations, we determined the inflammatory stress in the brain tissues of animals. Consistently, we found that MS extract promoted the expression of anti-inflammatory factor IL-4, and reversed the elevated inflammatory factors IL-1β/6 and TNF-α level in the brain tissue of LPS-induced depression-like rats. Recently, Song and colleagues unveiled that Ro25-6981, an antagonist of NR2B (glutamate inotropic receptor NMDA type subunit 2B), can alleviate LPS-related inflammation *via* reducing assembly of NR2B-CaMKII-PSD95 signal module in frontal cortex and hippocampus (Song et al., 2019). However, in our experiments, the NR2B-CaMKII-PSD95 signal module was not interfered after MS extract administration (data not shown). Also, we evaluated the regulatory effect of MS extract on NF-κB signaling pathway, which has been frequently evidenced involving the LPS-induced inflammatory stress *in vitro* (Kang et al., 2004; Zhang et al., 2018). We observed that MS extract inhibited the activation of NF-κB signaling by LPS treatment in BV-2 cells. Previously, the level of serum IL-10, a major immune regulatory cytokine exhibiting profound anti-inflammatory functions both in human and animal models, has been frequently observed a significant reduction in LPS-treated mice (Ohgi et al., 2013; Yao et al., 2015; Zhang et al., 2017). However, we found that the administration of LPS cannot down-regulated the expression of IL-10 in brain tissues, and MS treatment does not induce a distinguishable elevation of IL-10 mRNA level (data not shown). Collectively, we suppose MS extract may achieve its neuro-protective function in a NF-κB signaling dependent manner, of which exact mechanisms need to be further elucidated.

In conclusion, we have demonstrated that MS extract can alleviate the cognitive impairment of LPS-induced depression-like mice. It also exhibits excellent inhibitory effects on neuron apoptosis, astrogliosis and inflammatory stress *in vivo*. Given the edibility of MS, it may be widely used for food to relieve the symptoms of neuro-inflammation associated disorders such as depression.

Declaration of Competing Interest

The authors declare that they have no competing interests.

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